Impacts of Avidity and Specificity on the Antiviral Efficiency of HIV-1-Specific CTL[1]

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Although CD8$^+$ CTLs are presumed to be an important mediator of protective immunity in HIV-1 infection, the factors that determine CTL antiviral efficiency are poorly understood. Two factors that have been proposed to influence CTL antiviral function are antigenic avidity and epitope specificity. In this study we evaluate these by examining the activity of HIV-1-specific CTL against acutely infected cells. The ability of CTL to kill infected cells is variable and depends more on epitope specificity than functional avidity within the range for the tested clones (50% of maximal killing, 50 pg/ml to 100 ng/ml); killing efficiency is similar for different clones recognizing the same epitope, despite their variation in avidity. When CTL clones are tested for their ability to suppress viral replication, similar results are observed. Inhibition is more dependent on epitope specificity than functional avidity among the tested clones (50% of maximal killing, 20 pg/ml to 20 ng/ml). Thus, CTL specificity can be an overriding factor in the ability of CTL to interact with HIV-1-infected cells, indicating that factors determining the process of epitope presentation on infected cells have a key influence on CTL efficiency. These results suggest that CTL specificity may have a pivotal role in the immunopathogenesis of infection, and that simple quantitative measures of CTL may be insufficient indicators of the CTL response to HIV-1. *The Journal of Immunology*, 2003, 171: 3718–3724.

Major histocompatibility complex class I-restricted CTL are believed to have an important role in the immunopathogenesis of HIV-1 infection. Studies have shown the correlation of CTL responses to long-term nonprogressing infection (1, 2), control of viremia in acute (3, 4) and chronic (5) infection, and possibly protection from infection (6, 7). Further experimental evidence has come from the SIV macaque model, where depletion of CD8$^+$ cells has caused markedly elevated viremia (8–10). As such, there has been great interest in understanding the role of HIV-1-specific CTL in the control of infection and promoting such responses in vaccine strategies.

The ability to define and quantitate HIV-1-specific CTL has been markedly enhanced by recent technological advances. Previous assays, such as measuring virus-specific cytolytic activity of bulk PBMC by chromium release, and quantitating the frequency of CTL precursors by limiting dilution, have been supplanted by technically simpler and more precise assays such as ELISPOT, intracellular IFN-γ staining, and peptide-MHC tetramer binding (reviewed in Ref. 11). These new approaches have greatly simplified the definition of epitopes and drastically improved the ability to quantitate specific responses accurately. Despite the power of these methods to define the virus-specific CTL in HIV-1-infected persons and vaccinees, none of these assays directly reflects the antiviral potential of the CTL they detect (12). Indeed, such detailed characterizations of the breadth and magnitude of HIV-1-specific CTL have failed to show a clear relationship with viremia (13, 14).

Because standard techniques to detect HIV-1-specific CTL generally rely on recombinant or synthetic Ags to trigger responses (11), they do not take into account the efficiency of epitope presentation by HIV-1-infected cells. The efficiency of triggering of CTL and therefore recognition and clearance of infected cells depends on the efficiency of epitope processing and presentation through the class I pathway, and it is likely that different epitopes are not all equivalent in this respect (15, 16). Thus, the ability of CTL to interact with HIV-1-infected cells may be effected by virologic and cellular factors that are entirely substituted and bypassed by using synthetic peptides or recombinant Ags. Because the intracellular phase of viral replication is short (17, 18), the efficiency of CTL recognition may be a crucial determinant of CTL antiviral activity. In addition, most recent CTL assays rely on IFN-γ release or TCR labeling by peptide/MHC-I tetramers as a qualitative indicator of the effector capacity of CTL. Although the precise effector mechanisms whereby CTL suppress HIV-1 replication in vivo remain unclear (19), evidence suggests that these markers may be imperfect indicators of CTL functions such as cytolysis (20). Again, because the target cells for these techniques are not HIV-1-infected cells, the antiviral effects of CTL may not be determined by assays such as ELISPOT and tetramer binding.

These limitations have precluded a clear understanding of the factors that determine the antiviral efficiency of CTL. Most studies addressing this issue have been correlative, examining the relationship of CTL specificity and frequency to viral sequences and viremia. To understand better the antiviral properties of CTL, we have devised assays to evaluate the interaction of HIV-1-specific CTL clones with acutely HIV-1-infected cells (21, 22). In this study we apply these methods to evaluate the impacts of CTL

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specificity and functional avidity on the direct interaction of CTL with HIV-1, providing perspective on a poorly understood aspect of CTL antiviral function.

Materials and Methods

HIV-1 stocks

HIV-1 IIIB was generated as previously described (21, 22). A variant of NL4-3 (23) containing the consensus B sequence for the common p17 epitope SLYNTVATL was produced by point mutagenesis of the p83–2 plasmid (which contains the NL4-3 wild-type sequence SLYNTIAVL) (24). NL4-3.1 virus was then produced by electroporation of H9 cells with p83–2 variant and p83–10 plasmid DNA linearized with *Eco*R1 (25). Low passage virus stocks were frozen in aliquots at −80°C until use, and titered as previously described (26). The epitopes against which the CTL clones were derived were conserved in NL4-3.1 and IIIB, as confirmed by proviral DNA sequencing.

CTL clones

HIV-1-specific CTL clones were obtained from the blood of infected individuals by cloning of PBMC at limiting dilution, characterized for specificity and HLA-restriction, and maintained as previously described (27). Briefly, clonal cell lines were isolated from bulk or peptide-stimulated PBMC by culturing at limiting dilution, and clones responding to viral proteins were fine-mapped using successively truncated peptides. HLA restriction was then deduced by screening for activity against peptide-labeled partially HLA-matched B cell lines. The resulting clones (generally pure populations of $CD3^+CD8^+$ cells by flow cytometric analysis) were maintained with periodic restimulation using PHA or anti-CD3 Ab and irradiated allogeneic feeder PBMC. The clones used in these studies are listed in Table I.

Target cells

The cell line T1 (28) served as an HLA-matched target cell line for the A2- and B60-restricted CTL. H9 cells (29) were utilized as target cells for HLA B15 (Bw62)-restricted CTL. HLA B14-transfected H9 cells (H9-B14) (21) served as target cells for HLA B14-restricted CTL. HLA typing was performed at the Massachusetts General Hospital tissue typing laboratory. These cell lines were maintained as previously described (21, 22).

Chromium release assays

Target cells were uninfected or infected with HIV-1 at excess multiplicity of infection (≥3 tissue culture infectious doses per target cell) and utilized as target cells as previously described (21). Briefly, the cells were labeled with ^{51}Cr for use as target cells for CTL clones at an E:T ratio of 5:1 (5×10^4 CTL with 10^4 target cells per well in a 96-well U-bottom plate) in standard 4-h chromium release assays (after 4 days for acutely infected cells). Uninfected target cells were prelabeled with the appropriate synthetic epitope at 100 μg/ml or the indicated concentrations for peptide titrations. Controls included cells not labeled with peptide. Specific lysis was calculated as: [(experimental chromium release − spontaneous chromium release)/(maximal chromium release − spontaneous chromium release)] × 100.

Calculation of killing efficiency of infected cells

The percentage of HIV-1-infected cells after 4 days of infection was determined by intracellular staining and flow cytometric analysis as previously described (21). Briefly, the cells were fixed and then permeabilized with lysolecithin/nonionic detergent, followed by staining with fluorescent anti-p24 Ab and flow cytometric analysis. The efficiency of killing of infected cells (corrected for efficiency of infection) was calculated by: 100 × [(specific lysis of infected cells)/(specific lysis of excess cognate peptide labeled cells × the fraction of cells expressing intracellular p24 Ag)]. The percentage of infected cells was generally >70% in these assays (median of 97.6% for 11 independent experiments).

Coculture assays to measure viral inhibition

Acutely infected T1 cells were cocultured with CTL clones to measure viral inhibition, as previously described (22). Briefly, T1 cells were infected with 500 pg p24 of virus stock per 10^6 cells, followed by coculture with CTL clones. A total of 5×10^5 T1 cells were then cocultured with 1.25×10^5 CTL clone in a volume of 2 ml in a 24-well flat-bottom plate. At 2–3-day intervals, 1 ml of medium was removed for quantitative p24 Ag ELISA (DuPont, Boston MA) and replaced with 1 ml fresh medium. Inhibition in $\log_{10}$ units was calculated as: $-\log_{10}$(p24 with CTL/p24 without CTL) on day 7 of coculture.

Statistics

Statistical calculations were performed with Excel (Microsoft Corporation) on a G4 Macintosh computer.

Results

HIV-1-specific CTL clones of different specificities kill exogenously peptide-loaded cells with similarly high efficiency

Classical methods for detecting and isolating HIV-1-specific CTL clones select for cells with lytic activity (27). We screened multiple clones (isolated from the PBMC of HIV-1-infected persons) in parallel to evaluate the degree to which they could kill target cells loaded with excess epitope. Under the standardized conditions of excess peptide (100 μg/ml), RT-, Gag-, and Nef-specific CTL were similar in their killing of target cells (Fig. 1). Thus despite recognition of different epitopes by CTL, there was no difference in lytic potential. This indicated that the cytolytic potentials of CTL isolated for our studies were essentially equivalent, and that different clones possessed similar effector capacity, as measured by the chromium release assay.

Table I. *Functional avidity of CTL clones*[a]

Clone	HLA	Protein	Epitope	SD_{50} (pg/ml)
115p17-5B	A2	Gag 77–85 (p17)	SLYNTVATL (Gag/p17-SL9)	1,000
18030D23	A2	Gag 77–85 (p17)	SLYNTVATL (Gag/p17-SL9)	1,000
161JxA14	A2	Gag 77–85 (p17)	SLYNTVATL (Gag/p17-SL9)	20,000
161JD27	B60	Gag 92–101 (p17)	IEIKDTKEAL (Gag/p17-IL10)	8,000
15160A49	B14	Gag 166–174 (p24)	DRFYKTLRA (Gag/p24-DA9)	100,000
161JxA12	B60	Gag 176–184 (p24)	SEGATPQDL (Gag/p24-SL9)	30
68A62	A2	Pol 309–317 (RT)	ILKEPVHGV (RT-IV9)	50
14142.11	A2	Pol 309–317 (RT)	ILKEPVHGV (RT-IV9)	20,000
115K4	B14	Env 584–592 (gp41)	ERYLKDQQL (Env EL9)	10,000
15160DC4	B14	Env 584–592 (gp41)	ERYLKDQQL (Env EL9)	60,000
18030B31	B14	Env 584–592 (gp41)	ERYLKDQQL (Env EL9)	40,000
LWF C8	B14	Env 584–592 (gp41)	ERYLKDQQL (Env EL9)	5,000
KM3	B60	Nef 92–100	KEKGGLEGL (Nef KL9)	30
STD11	B60	Nef 92–100	KEKGGLEGL (Nef KL9)	20

[a] For each clone is given the recognized minimal epitope (amino acid numbering according to the HXB2 sequence), HLA restriction, and SD_{50} value. The range of SD_{50} values was 20–100,000 pg/ml; median 6,500 pg/ml.

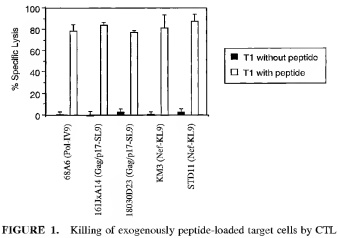

FIGURE 1. Killing of exogenously peptide-loaded target cells by CTL clones. CTL clones were tested for killing of HLA-matched T1 target cells preloaded with 100 μg/ml of cognate peptide by chromium release assay (5:1 E:T ratio). The means for three independent experiments (from three separate passages) are indicated. The error bars represent one SD. The SD_{50} values for these clones ranged from 20 pg/ml for STD11 to 20 ng/ml for 161JxA14 (Table I). In our general experience, clones maintaining stable lytic activity and proliferating over multiple consecutive passages tend to be similar in their killing of peptide-loaded target cells by chromium release assay, as seen in this controlled comparison of five clones.

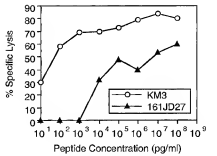

FIGURE 2. Determination of functional avidity of CTL clones. CTL clones were evaluated for functional avidity by peptide titration chromium release assays with autologous EBV-transformed B cell targets. Examples of two clones are shown. Between repeated assays for specific clones, SD_{50} values were reproducible within 10-fold (data not shown).

Individual CTL clones vary in antigenic avidity over several orders of magnitude

Having demonstrated the equivalent lytic potential of CTL under standard conditions of excess epitope, we next evaluated the sensitivity of clones to triggering by the target epitope, or "functional avidity" (30). Avidity was measured in terms of the sensitizing dose of peptide required for 50% of maximal killing (SD_{50}),[3] by standard peptide titration chromium release assays (Fig. 2, Table I). The clones varied in SD_{50} over almost four orders of magnitude, ranging from ~20 pg/ml to 100 ng/ml. Notably, even clones recognizing the same epitope could vary by 400-fold in their functional avidity, such as 68A62 (SD_{50}, 50 pg/ml) and 14142.11 (20 ng/ml). Thus despite equivalent lytic potential among clones (at excess peptide concentration), they varied greatly in sensitivity to triggering by epitope. These results indicated that CTL functional avidity is highly variable and is not directly related to epitope specificity.

The efficiency of infected cell killing varies among individual clones, and appears to be associated with specificity and not avidity

Functional avidity has been proposed to influence the antiviral pressure exerted by CTL in vivo (31), and we next evaluated whether avidity effects the ability of CTL to kill acutely infected cells. Because the use of exogenously added peptides bypasses the physiologic process of HIV-1 protein expression and therefore likely effects the magnitude and kinetics of epitope presentation by MHC-I on the cell surface, several CTL clones were tested for their ability to kill acutely target cells after acute high multiplicity HIV-1 infection (Fig. 3). The efficiency of infected cell killing (observed percentage of killing adjusted for the percentage of infected cells and maximal killing of peptide-labeled controls) was not clearly related to the functional avidity of the clones over the SD_{50} range from 50 pg/ml to 100 ng/ml (Fig. 3*A*); higher avidity (lower SD_{50}) did not result in higher efficiency of infected cell killing over this range. When specificity was considered, however, it was clear that clones recognizing particular epitopes in RT and Env were consistently less efficient than those recognizing an epitope in Gag (Fig. 3*B*). This was despite variation in functional avidity among the individual clones. Specifically, it was notable that a more avid RT-specific clone was not more efficient than a less avid clone recognizing the same epitope (Fig. 3*A*, 68A62 vs 14142.11), and that two Gag-specific clones were more efficient

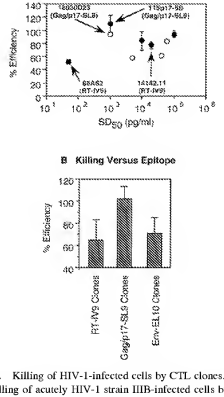

FIGURE 3. Killing of HIV-1-infected cells by CTL clones. CTLs were tested for killing of acutely HIV-1 strain IIIB-infected cells by chromium release assay. *A*, The efficiency of infected cell killing is plotted against SD_{50}. Mean values (●) are indicated for clones that were multiply tested (115p17-5B, 15160A49, 68A62, 14142.11, 115K4), with error bars representing one SD. Values for clones (○) are indicated that were tested once (18030D23, 15160DC4, 18030B31, LWF C8). Arrows indicate some specific clones of interest (see in *Results*). *B*, The mean efficiencies for multiple clones recognizing the same epitopes are plotted. Two clones recognized an RT epitope (68A62 and 14142.11), two recognized a Gag epitope (115p17-5B and 18030D23), and four recognized an Env epitope (115K4, 15160DC4, 18030B31, and LWF C8). Error bars represent one SD.

[3] Abbreviation used in this paper: SD_{50}, 50% of maximal killing.

than an RT-specific clone of higher avidity (Fig. 3A, 18030D23 and 115p17–5B vs 68A62). Thus, the ability to kill infected cells appeared to be related to epitope specificity and not avidity, over the range of SD_{50} values tested (≤100 ng).

The suppression of HIV-1 replication by these CTL clones is also related to specificity more than avidity

We have previously identified the importance of cytolysis in the ability of CTL to suppress HIV-1 replication (19). Using the same coculture system, we next evaluated the impacts of functional avidity and specificity on the antiviral activity of CTL by directly measuring the ability of panels of CTL clones to inhibit viral replication (Figs. 4 and 5). When multiple clones were concurrently measured for HIV-1 suppression, there was no clear correlation of avidity with antiviral activity (Figs. 4A and 5A) for SD_{50} values ranging over three orders of magnitude (20 pg/ml to 20 ng/ml). Although the two most inhibitory clones had the highest functional avidity (SD_{50}, 20 pg/ml and 30 pg/ml), the least inhibitory clone had similar avidity (50 pg/ml). When inhibitory activity was compared with epitope specificity, however, viral suppression was similar among different clones recognizing the same epitope (Fig. 4B).

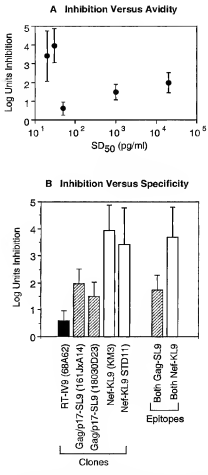

FIGURE 4. Suppression of HIV-1 replication by RT-, Gag-, and Nef-specific CTL. The indicated CTL clones were tested for their ability to suppress HIV-1 replication in coculture with acutely infected cells (HIV-1 NL4-3.1-infected T1 cells). Viral p24 Ag concentrations in the presence and absence of CTL were used to calculate the degree of viral suppression in log_{10} units after 7 days of culture. Inhibition was evaluated in quadruplicate for each clone (mean ± one SD). *A*, Inhibition is plotted against SD_{50} for each clone. *B*, Inhibition is plotted for each clone (first five bars), and mean inhibition for the Gag- and Nef-specific clones recognizing the same epitopes is plotted (last two bars).

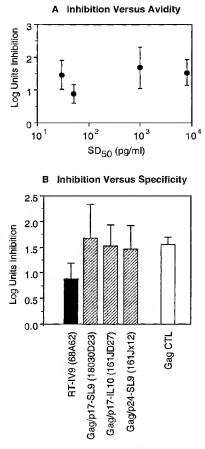

FIGURE 5. Suppression of HIV-1 replication by RT- and Gag-specific CTL. The indicated CTL clones were tested for their ability to suppress HIV-1 replication in coculture with acutely infected cells (HIV-1 IIIB-infected T1 cells). The mean inhibition (after 7 days of culture) from multiple concurrent experiments (at least 6 separate concurrent comparisons using independently infected target cells) was calculated for each clone. *A*, Inhibition is plotted against SD_{50} for each clone. *B*, Inhibition is plotted for each clone (first four bars), and mean inhibition for the three Gag-specific clones recognizing different epitopes is plotted (last bar).

Gag-specific clones recognizing three different epitopes in p17 and p24 also suppressed virus similarly, and were consistently more inhibitory than an RT-specific clone (Fig. 5B). Interestingly, two Gag-specific clones recognizing the same epitope but differing 20-fold in avidity were similar in their ability to suppress viral replication (Fig. 4B, 161JxA14 vs 18030D23), and both were more suppressive than an RT-specific clone that was 20- to 400-fold more avid (Fig. 4B, 171JxA14 and 18030D23 vs 68A62). These data strongly suggested that specificity plays a dominant role in determining the antiviral activity of CTL.

Discussion

Although HIV-1-specific CTL are believed to be an important protective immune response, the precise determinants of efficacy against HIV-1 remain poorly defined. Evidence suggests that the control of viral replication in vivo is not a simple function of quantity as reflected by frequency of virus-specific γ-IFN-producing cells (13), suggesting that qualitative factors may be involved. Importantly, potential differences in the antiviral activity of CTL remain to be determined. Because most commonly used CTL assays allow detection of HIV-1-specific CTL but not measurement of antiviral function, speculation concerning these factors has been based largely on correlative data. Two factors proposed to affect the ability of CTL to control HIV-1 are epitope specificity and

CTL avidity for Ag. In SIV-infected macaques, CTLs in early infection, which tend to recognize early expressed viral proteins such as Tat and Nef, induce more escape mutations than late CTL (32, 33). This suggests the possibility that CTL recognizing certain epitopes exert greater immune pressure than others. Less direct evidence for this phenomenon also exists for HIV-1, where early CTL also appear to target early viral proteins more commonly than CTL in chronic infection (34). Although not systematically evaluated as for SIV, escape mutation also appears to occur more consistently during acute infection than during chronic infection (3, 35). The SIV model also has raised the possibility that functional avidity has an important role. It appears that early CTL are higher avidity, and that higher avidity is correlated with the greater escape (possibly due to greater immune pressure) induced by these CTL (31). These trends remain to be demonstrated in HIV-1 infection.

In this study, we evaluate the impacts of functional avidity and specificity by comparing directly multiple CTL clones in assays that reflect the direct interaction of CTL with acutely infected cells. By testing the ability of CTL clones recognizing epitopes in multiple HIV-1 proteins to kill infected cells, we do not detect an effect of avidity on the efficiency of infected cell recognition. This holds true over the range of avidity for the clones we tested (spanning nearly four orders of magnitude), suggesting that a functional avidity of SD_{50} 100 ng/ml or better is not limiting for CTL function against infected cells. Although we did not evaluate directly the influence of epitope binding affinity for MHC-I, our finding that RT-IV9-specific CTL are less efficient killers of infected cells than Gag/p17-SL9-specific CTL suggests that this is not the key factor, because IV9 has much higher binding affinity for A*02 than SL9 (36). However, clones of the same epitope specificity (despite their variation in SD_{50}) are similar in their ability to kill infected cells, indicating that specificity is an overriding determinant of function. Our clones recognizing the same epitope had distinct TCR sequences and variable chain usages (Ref. 37 and data not shown), indicating that TCR differences affected avidity and antiviral efficiency differentially.

These results extend previous studies indicating functional differences between CTL recognizing different epitopes. Earlier work demonstrated a marked difference between presentation of A2-restricted HIV-1 RT (IV9) and Gag/p17 (SL9) epitopes, presented on infected cell surfaces at an average of 12 and 400 copies, respectively (15). CTL targeting the RT-IV9 epitope were less efficient than those targeting the Gag/p17-SL9 epitope (15, 21), suggesting that epitope presentation may be the overriding factor, and that avidity plays less of a role because TCR binding serves as a yes/no trigger for the effector functions of CTL. Although one might expect that avidity could effect triggering under conditions of limiting epitope, the higher avidity clone recognizing the IV9 epitope (68A62; SD_{50}, 50 pg/ml) is consistently less efficient than the lower avidity clone (14142.11; SD_{50}, 20 ng/ml), suggesting that sensitivity of the TCR is not a limiting factor for CTL recognition of infected cells by our clones.

We generalize these observations to viral inhibition by CTL. In controlled comparisons of panels of CTL clones, there is again no apparent impact of functional avidity on this measurement of antiviral function. Although the two most inhibitory clones have the highest avidity (SD_{50}, 20 pg/ml and 30 pg/ml), another clone with similar avidity is consistently the least inhibitory. Over the range of SD_{50} evaluated (20 pg/ml to 20 ng/ml), avidity appears to have little impact on the ability of CTL clones to suppress viral replication, although in a recent study of viral escape from CTL, we have found that viruses containing epitope mutants recognized with SD_{50} >400 ng/ml are not inhibited (24). When the specificity of the clones is considered, the suppressive activity of the clones is similar among CTL of the same specificity. There is a clear trend that an RT-specific clone is the least effective, several Gag-specific clones are intermediate, and two Nef-specific clones are the most highly suppressive, despite the wide variability in avidity. These results agree with the data on efficiency of infected cell killing, and confirm and extend our earlier findings with CTL recognizing the RT-IV9 and Gag/SL9 epitopes (22).

Thus in the present study, two specific observations using CTL clones recognizing the RT-IV9 and Gag/p17-SL9 epitopes hint that avidity plays a small role in the interaction of CTL with HIV-1-infected cells (for the range of SD_{50} of the clones we studied). We find that clones recognizing the same epitope behave similarly despite varying in avidity by up to 400-fold. Moreover, we note that clones recognizing one epitope (Gag/p17-SL9) are consistently more active against HIV-1 than clones recognizing another epitope (RT-IV9), despite being up to 200-fold less avid. These findings suggest that avidity may be a lesser factor in the interaction of these CTL with HIV-1.

Unexpectedly, the Nef-specific CTL clones are even more efficient inhibitors of viral replication than the Gag-specific CTL. Because we have already shown that the killing of infected cells by SL9-specific CTL approaches 100% efficiency, this suggests that other properties besides protein expression levels are responsible for the superior efficiency of Nef-specific CTL. The superior inhibition by the Nef-specific CTL appears unrelated to HLA B60-restriction, as two B60-restricted Gag-specific CTL are similar to SL9-specific CTL. Epitope specificity therefore appears to be the determining factor. Moreover, we have observed recently that Nef- and Gag-specific CTL functionally differ in their selection of epitope escape mutations under conditions of incomplete viral suppression in vitro (24). These findings therefore indicate that the efficiency of infected cell killing is not the only determinant of antiviral efficiency of CTL. Factors such as the kinetics of epitope expression may also have a major impact, perhaps through kinetic differences in protein expression (38).

As a whole, these data suggest that functional avidity (over the four log range for the tested clones) does not appear to affect the antiviral function of CTL by comparison to epitope specificity, which appears to be a major determinant. This implies that epitope presentation has a crucial role in the antiviral efficiency of CTL. Quantitative and kinetic differences in the expression of RT, Gag, and Nef may be important determinants of this phenomenon. In the context of what is known about differential Gag-Pol translation (39), higher levels of Gag may lead to excess Gag epitope and limiting RT epitope presentation at the cell surface. Furthermore, Tat, Rev, and Nef are the earliest proteins produced by infected cells (38), and earlier presentation of Nef epitopes could therefore be advantageous for Nef-specific CTL, given the narrow temporal window between potential CTL clearance and virion production by acutely HIV-1-infected cells (21).

A caveat that should be noted, however, is that functional avidity (SD_{50}) is not a direct measure of TCR affinity for the peptide-MHC complex (40). Although TCR affinity is a key component, the efficiency of the signal transduction machinery may also contribute to avidity, modifying it by 50-fold or more during "functional avidity maturation" of CTL in vivo (40). Thus our SD_{50} measurements are an indirect indicator of CTL sensitivity for Ag, and we cannot entirely exclude a direct role for TCR affinity (or epitope and MHC-I binding affinity, as previously discussed). Still, TCR affinity is probably the major determinant of avidity, and our data are pertinent to observations in the SIV model, in which correlations have been made in terms of SD_{50} values (31).

Also, our findings do not exclude the contribution of other factors to CTL efficiency. Evidence presented by Shankar et al. (41)

has demonstrated that CTL recognizing another RT epitope appear to be 100% efficient at lysing infected cells. This contradictory finding may reflect a methodological difference (HIV-1-infected target cells in that study were enriched by negative selection for CD4 down-regulated cells). Alternatively, this result could suggest that there are other determinants of epitope presentation efficiency besides protein expression levels, such as differences in processing, transport, or binding (16). Most likely, the influence of epitope specificity on the antiviral efficiency of CTL is subject to modulation by multiple factors.

Finally, the relationship of our results to the antiviral efficiency of CTL in vivo remains to be determined. Of note, experiments in a murine model have suggested that avidity is an important determinant of CTL suppression of viruses in vivo (30). Different CTL that were similarly able to kill virus-infected cells in vitro were found to be variably effective against virus after adoptive transfer into mice, corresponding to avidity, in contrast to our findings. A potential explanation for this apparent discrepancy is that Alexander-Miller et al. (30) studied murine CTL with SD_{50} values ranging from ~100 ng/ml to 10 μg/ml, whereas our CTL ranged from 20 pg/ml to 100 ng/ml. Thus, a threshold of avidity may be required for antiviral function, and specificity may play an additional modulatory role for CTL achieving that threshold.

In conclusion, these data implicate CTL specificity as a key factor in the ability of CTL to control HIV-1 replication. This implies that the targeting of CTL in vivo may have a role in immunopathogenesis and vaccine efficacy. Although routine assays for CTL are useful in describing CTL specificity and frequency, these assays do not distinguish the relative efficiencies of different CTL against HIV-1. Further mechanistic studies will be required to elucidate the precise determinants of CTL antiviral efficacy in vivo. A clearer understanding of these factors may have important implications in strategies for immunotherapy and vaccine development.

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